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HUMAN METHIONINE SYNTHASE REDUCTASE: CLONING,
AND METHODS FOR EVALUATING RISK OF NEURAL TUBE
DEFECTS, CARDIOVASCULAR DISEASE, AND CANCER

Roy A. Gravel et al.

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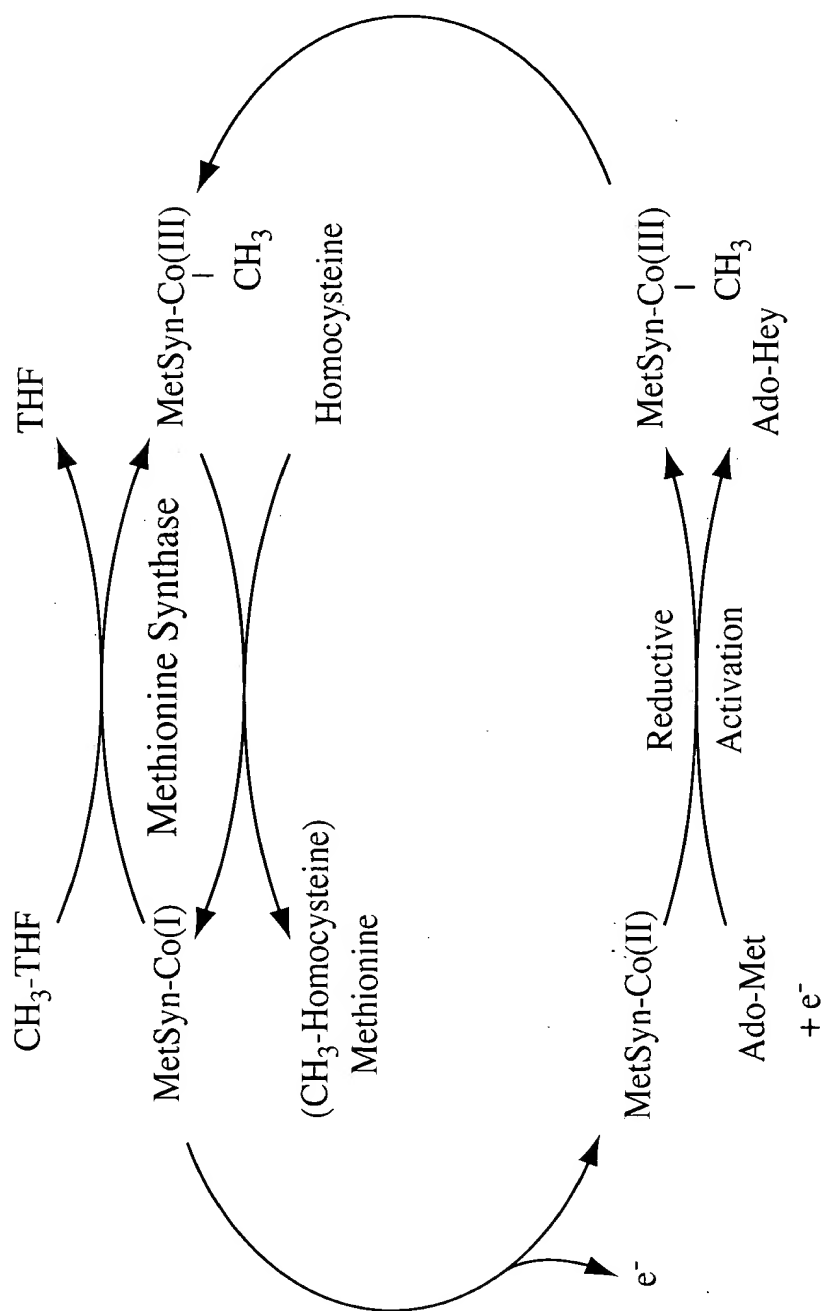


Fig. 1

PRIMERS	SEQUENCE	LOCATION
\overrightarrow{V} $\overleftarrow{1103A}$	5' - CTCCTGCTCGAACATCTTCCTAA	1318 - 1341
\overrightarrow{V} $\overleftarrow{1103A}$	5' - AATAGATAATCCCTATCCTTATGCC	1766 - 1742
\overrightarrow{V} $\overleftarrow{1803E}$	5' - CCCTGGCTCCTAAGATATCCATC	1544 - 1566
\overrightarrow{V} Δ $\overleftarrow{1103A}$	5' - CGAACACAAATCTTTCCACTTACC	1573 - 1598
	5' - CAAGTTGGTGAAGTCGCGTTG	-79 - -57
	5' - ATGCCCTTGAAGTGATGAGGAGTTT	-13 - 12
	5' - TTCTTACAACATAGAGAGAACTC	1663 - 1686
	5' - TTGCACAAGGCATCATGTACATC	1998 - 1975
	5' - AAACCTCCTCATCACTTCAAGGCAT	12 - -13
	5' - CTTGCACACGAATATGGTCTGGG	1370 - 1348
	5' - TGGCATCACCTGCATCCTTGAGG	506 - 528
	5' - GATGTACCTGTAAATATTCTGGGGG	760 - 736
	5' - AATCCACGGCTCAACCACAATTC	429 - 406
	5' - CTCGAAATTAACCTCCTCACTAAAGGG	in Bluescript
	5' - AACCCATACCGCAGGTGAGCAA	278 - 256
	5' - TTTAGTACTTTTCAGTCAAAAAGCTTAAT	2148 - 2120
	5' - ATAAACGACTTCAAGAGCTTGGAGC	335 - 359
	5' - AGGTTTGGCACTAGTAAAGCTGACT	2173 - 2149



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CAAGTTGGTGAAGTCGGCTGTGTCAGGTTCTGTCGCCGGCTGGCGGGGGTGTTCACGTGTACATGCGCTTGAAGTG
1 ATGAGGAGGTTCTGTACTATATGCTACACAGGAGGAGCGCAATCGCAGAAGAAATGTGTGAGCAAGCTGTGGTACATGGATTTCTGCAGATCTTCACTGTATTAGTGAA
M R R F L L L Y A T Q Q G Q A K A I A E M C E Q A V V H G F S A D L H C I S E 40
121 TCCGATAAGTATGACCTAAACCGAAACAGCTCTCTTGTGTGTGTCTTACACGGGACCGGAGACCCACCCACACAGCCCGCAAGTTTGTAAAGAAATACAGAACCAACA
S D K Y D L K T E T A P L V V V S T T G T G D P P D T A R K F V K E I Q N Q T 80
241 CTGCCGGTTGATTTCTTGTCTACCTCGCGTATCGGTACTGGGTACAGAAATACACCTACTTTTGCATGGGGGAAGATAATTGATAAACGACTTCAAGAGCTTGGAGCC
L P V D F F A H L R Y G L L G L G D S E Y T Y F C N G G K I I D K R L Q E L G A 120
361 CGGCATTCTATGACACTGGACATGCAGATGCTGTAGTTTGAACCTGTGTGAGCCGTGGATTCTGGACTCTGGCCACCCCTCAGAAAGCATTTTAGGTCAAGCAGAGGACAA
R H F Y D T G H A D D C V G L E L V V E P W I A G L W P A L R K H F R S S R G Q 160
481 GAGGAGATAAGTGGCGCACTCCCGGTGGCATCACTGCAATCCTTGAGGACAGACCTGTGAACTCAGAGCTGTACACATTGAATCTCAAGTCGAGCTTCTGAGATTGCGATTCAGGA
E E I S G A L P V A S P A S L R T D L V K S E L L H I E S Q V E L L R F D D S G 200
601 AGAAGGATTCTGAGTTTGAAGCAAAATGCAGTGAACAGCAACCAATCCAATGTTAATTGAAGACTTTGAGTCTCTCACTTACCCGTTCCGTACCCCACTCTCAAGCCTCTCTG
R K D S E V L K Q N A V N S N Q S N V I E D F E S S L T R S V P P L S Q A S L 240
721 AATATTCCTGGTTTACCCCAAGATATTTACAGGTACATCTGCGAGAGTCTCTTGGCCAGGAGAAAGCCAAAGTATCTGTGACTTCAGCAGATCCAGTTTTCAGTGCCCAATTTCAAAG
N I P G L P P E Y L Q V H L Q E S L G Q E E S Q V S V T S A D P V F Q V P I S K 280
841 GCAGTCACTTACGAATGATGCCATAAAACCACTCTGCTGTAGATTGGACATTTCAANTACAGACTTTTCTATCAGCTGGAGATGCCCTTCAGCGTGATCTGCCCTAACAGT
A V Q L T T N D A I K T T L L V E L D I S N T D F S Y Q P G D A F S V I C P N S 320
961 GATTCTGAGGTACAAAGCCTACTCCAAAGACTGCAGCTTGAAGATAAAGAGAGCACTGCGTCTTTTGAATAAAGGCAGACACAAAGAGAGGAGTACTTACCCCAAGCATATA
D S E V Q S L L Q R L Q L E D K R E H C V L L K I K A D T K K G A T L P Q H I 360
1081 CCTGCGGGATGTTCTCTCCAGTTCAATTTTACCTGGTGTCTTGAATCCGAGCAATTCCTAAAGGCAATTTTTCGAGCCCTTGTGGACTATACAGTGACAGTGTGTAAGCGCAGG
P A G C S L Q F I F T W C L E I R A I P K K A F L R A L V D Y T S D S A E K R R 400
1201 CTACAGGAGCTGTGCAGTAACAAGGGCAGCCGATTATAGCCGTTTGTACGAGATGCCCTGCTGTGGATCTCCTCGCTTCCCTTCTTGGCCAGCCACCACTCAGTCTC
L Q E L C S K Q G A A D Y S R F V R D A C A C L L L D L L L A F P S C Q P P L S L 440

Fig. 3-1

Fig. 3-2





Fig. 3-4



HUMAN METHIONINE SYNTHASE REDUCTASE: CLONING,
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HsMTRR	-----	
CeMTRR	-----	
HsCPR	MGDSHVDTSSTVSEAVAEVSLFSMTDMILFSLIVGLLTYWFLFRKKKEE	50
	FMN	
HsMTRR	-----MRRFLLLYATQQGQAKAIAEEMCE	24
CeMTRR	-----MTDFLIAFGSQTGQAETIAKSLKE	24
HsCPR	VPEFTKIQTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFANRLSK	100
 * * * * . .	
	FMN	
HsMTRR	QAVVHGFSADLHCISESDK-YDLKT-----ETAPLVVVVSTTGTGDP	66
CeMTRR	KAELIGLTPRLHALDENEEKFNLE-----EKLC-AIVVSSTGDGDAP	66
HsCPR	DAHRYG---MRGMSADPEEYDLADLSSLPEIDNALVVFCMATYGECDPT	146
	* * * * * *	
	FMN	
HsMTRR	DTARKFVKEIQNTLPVDFFAHLRYGLLGLGDSFYTYFCNGGKIIDKRLQ	116
CeMTRR	DNCARFVRRINRNSLENEYLKNLDYVLLGLGDSNYSSYQTIPRKIDKQLT	116
HsCPR	DNAQDFYDWLQETDVD---LSGVKFAVFLGNGKTYEHFNAMKGYVDKRLE	193
	* * * * * * * * *	
HsMTRR	ELGARHFYDTGHADDCVGLLELVPEWPIAGLWPALRKHFSSRGQEEISGA	166
CeMTRR	ALGANRLFDRAEADDQVGLLEVEPEWIEKFFATLASRFDISADKMN----	162
HsCPR	QLGAQRIFELGLGDDDGNEEDFITWREQFWPAVCEHF-----GV	233
	*** * * * * *	
HsMTRR	LPVASPASLRDVLKSELLHIESQVELL--RFDDSGRKDSEVLKQNAVNS	214
CeMTRR	-AITESSNLKLNQVKTE----EEKKALLQKRIEDEESDDEGRGRVIGID-	206
HsCPR	EATGEESSIRQYEL-----VVHTDIDAAKVVMGEMGRLLKSYEN	271
	
HsMTRR	NQSNVVEDF---ESSLTRSVPLS-QASLNIPGLPPEYLQVHLQESLGQ	260
CeMTRR	---MLIPEHYDYPEISLLKGSQTLSDNENLRVPIAPQPFIVSSVSNRKLP	253
HsCPR	QKP-----PFD-----AKNPFLAAVTNTRKLN	293
	
HsMTRR	EESQVS-----VTSADPVFQVPISKAVQLTT--NDAIKTTLLVELDIS	301
CeMTRR	EDTKLEWQNLCKMPGVVTKPFVLLVVSFAEFVTDPFSSKIKTKRMITVDFG	303
HsCPR	QGTE-----RHLMHLELD	306
	
HsMTRR	N--TDFSYQPGDAFSVICPNSDSEVQSLLQR-LQLEDKREHCVLLKIKAD	348
CeMTRR	DHAAELQYEPGDALYFCVPNPALVNFILKRCGVLDIADQQCEL-SINPK	352
HsCPR	ISDSKIRYESGDHVAVYPANDSALVNQLGK---ILGADLD--VVMSSLNNL	351
	. * * * * *	
HsMTRR	TKKKGATLPQHYPAGCSLQFIFTWCLEIRAIKKAFLRALVDYTSDSAEC	398
CeMTRR	TEKINAQIPGHVHKITTLRHMTTCLDIRRAPGRPLIRVLAESTSDPNEK	402
HsCPR	DEESNKKHP--FPCPTSYRTALTYLDITNPRTNVLVELAQYASEPSEQ	399
	* . . * * * * *	

Fig. 4-1



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HsMTRR	RRLQEL--CSKQGAADYSRFVRDACACLLDLLLAFPPSCQPPLSLLLEHLP	446
CeMTRR	RRLLEL--CSAQGMKDFTFVVRTPGLSLADMLFAFPNVKPPVDRLELLP	450
HsCPR	ELLRKMASSSGEGKELYLSWVVEARRHILAILQDCPSLRPPIDHLCELLP	449
	* . * . * . * . * * * * *	
	<u>FAD</u> <u>FAD</u> <u>FAD</u>	
HsMTRR	KLQPRPYSCASSSLFHPGKLHFVFNIVEFLSTATTEVLRKGVCTGWLALL	496
CeMTRR	RLIPRPYSMSS---YENRKARLIYSEMEFPATDGRRHRSRKGLATDWLNSL	497
HsCPR	RLQARYYSIASSSKVHPNSVHICAVVVEYETKAGR--INKGVATNWL---	494
	. * * * * . * . . * * . * *	
HsMTRR	VASVLQPNIHASHEDSGKALAPKISISPRTTNSFHLP-----DDPSIP	539
CeMTRR	R-----IGDKVQVLGKEPARFRLPPLGMTKNSAGKLP	529
HsCPR	RAKE-----PVGENGGRALVPMFVRKSQFRLPFK-----ATTP	527
	* *	
	<u>NADPH</u>	
HsMTRR	IIMVGPGTGIAPFIGFLQHREKLQEHPDGNFGAMW-LFFGCRHKDRDYL	588
CeMTRR	LLMVGPGTGVSVFLSFLHFLRKLKQDSPSDFVDVPRVLFFGCRDSSVDAI	579
HsCPR	VIMVGPGTGVAPFIGFIQERAWLRQQGKE--VGETLLYYGCRRSDEDYL	574
	..*****. * . * . * .. * . * * *	
	<u>NADPH</u> <u>NADPH</u>	
HsMTRR	FRKELRHFLKHGILTHLKVVSFSRDAPVGEEEAAPAKYVQDNIQLHGQQVAR	638
CeMTRR	YMSELEMFVSEGILTDLIICESEQ-----KGERVQDGLRKYLDKVL	621
HsCPR	YREELAQFHRDGAQTQNLVAFSRE-----QSHKVYVQHLLKQDREHLWK	618
	. * * * * * * * * * * * *	
	<u>NADPH</u>	
HsMTRR	ILLQE-NGHIYVCGDAKNMAKDVEDALVQIISKEVGVEKLEAMKTLATLK	687
CeMTRR	FLTASTESKIFICGDAKGMSKDVWQCFSDIVASDQGIPLDLEAKKKLMDLK	671
HsCPR	LI--EGGAHIYVCGDARNMARDVQNTFYDIVAELGAMEHAQAVDYIKKLM	666
	. * . * * * . * . * * . * . . . * . *	
	<u>NADPH/FAD</u>	
HsMTRR	EEKRYLQDIWS	698
CeMTRR	KSDQYIEDVWG	682
HsCPR	TKGRYSLDVVS	677
	* * *	

Fig. 4-2



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HsMTRR	-----	
CeMTRR	-----	
HsCPR	MGDSHVDTSSTVSEAVAEVSLFSMTDMILFSLIVGLLTYWFLFRKKKEE	50
	FMN	
HsMTRR	-----MRRFLLLYATQQGQAKAIAEEMCE	24
CeMTRR	-----MTDFLIAFGSQTGQAETIAKSLKE	24
HsCPR	VPEFTKIQTLTSSVRESSFVEKMKKTGRNIIVFYGSQTGTAEFANRLSK	100
 * * * * . .	
	FMN	
HsMTRR	QAVVHGFSADLHCISESDK-YDLKT-----ETAPLVVVVSTTGTGDPP	66
CeMTRR	KAELIGLTPRLHALDENEEKFNLE-----EKLC-AIVVSSTGDGDAP	66
HsCPR	DAHRYG----MRGMSADPEEYDLADLSSLPEIDNALVVFVCMATYGEDPT	146
	* * * * * *	
	FMN	
HsMTRR	DTARKFVKEIQNTLPVDFFAHLRYGLLGLGDSEYTYFCNGGKIIDKRLQ	116
CeMTRR	DNCARFVRRINRNSLENEYLKNLDYVLLGLGDSNYSSYQTIPRKIDKQLT	116
HsCPR	DNAQDFYDWLQETDVD---LSGVKFAVFLGNGKTYEHFNAMKYVDKRLE	193
	* * * * * * * * *	
HsMTRR	ELGARHFYDTGHADDCVGLELVVEPWIAGLWPALRKHFSSRGQEEISGA	166
CeMTRR	ALGANRLFDRAEADDQVGLELEVEPWIEKFFATLASRFDISADKMN----	162
HsCPR	QLGAQRIFELGLGDDDNLEEDFITWREQFWPAVCEHF-----GV	233
	*** . . . * * * * . . . *	
HsMTRR	LPVASPASLRDVLKSELLHIESQVELL--RFDDSGRKDSEVLKQNAVNS	214
CeMTRR	-AITESSNLKLNQVKTE----EEKKALLQKRIEEDSDDEGRGRVIGID-	206
HsCPR	EATGEESSIRQYEL-----VVHTDIDAAKVVMGEMGRLKSYEN	271
	
HsMTRR	NQSNVVIEDF---ESSLTRSVPLS-QASLNIPGLPPEYLQVHLQESLGQ	260
CeMTRR	---MLIPEHYDYPEISLLKGSQTLSNDENLRVPIAPQPFIVSSVSNRKLP	253
HsCPR	QKP-----PFD-----AKNPFLAAVTNTRKLN	293
	
HsMTRR	EESQVS-----VTSADPVFQVPISKAVQLTT--NDAIKTTLLVELDIS	301
CeMTRR	EDTKLEWQNLCKMPGVVTKPFEVLVVSAAEFVTDPFSSKIKTKRMITVDFG	303
HsCPR	QGTE-----RHLMHLELD	306
	
HsMTRR	N--TDFSYQPGDAFSVICPNSDSEVQSLLQR-LQLEDKREHCVLLKIKAD	348
CeMTRR	DHAAELQYEPGDAIYFCVPNPALEVNFILKRCGVLDIADQQCEL-SINPK	352
HsCPR	ISDSKIRYESGDHVAVYPANDSALVNQLGK---ILGADLD--VVMSSLNNL	351
	. * * * * *	
HsMTRR	TKKKGATLPQHIPAGCSLQFIFTWCLEIRAIPKKAFLRALVDYTSDSA EK	398
CeMTRR	TEKINAQIPGHVHKITTLRHMTTCLDIRRAPGRPLIRVLAESTSDPNEK	402
HsCPR	DEESNKKHP--FPCPTSRYTALTYILDITNPRTNVLVELAQYASEPSEQ	399
	* . . * * * * . . . * *	

Fig. 4-3



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HsMTRR	RRLQEL--CSKQGAADYSRFVRDACACLLDLLLAFSPCQPPLSLLLEHLP	446
CeMTRR	RRLLEL--CSAQGMKDFDFVTRTPGLSLADMLFAFPNVKPPVDRLELLP	450
HsCPR	ELLRKMASSSGEGKELYLSWVVEARRHILAILQDCPSLRPPIDHLCCELLP	449
	* . * . * . * . * . * . * . * . *	
	FAD FAD FAD	
HsMTRR	KLQPRPYSCASSSLFHPGKLHFVFNIVEFLSTATTEVLRKGVCTGWLALL	496
CeMTRR	RLIPRPYSMSS---YENRKARLIYSEMEFPATDGRRHRSRKGGLATDWLNSL	497
HsCPR	RLQARYYSIASSSKVHPNSVHICAVVVEYETKAGR--INKGVATNWL---	494
	. * * * * . * . . * . * . *	
HsMTRR	VASVLQPNHASHEDSGKALAPKISISPRTTNSFHLP-----DDPSIP	539
CeMTRR	R-----IGDKVQVLGKEPARFRLPPLGMTKNSAGKLP	529
HsCPR	RAKE-----PVGENGGRALVPMFVRKSQFRLPFK-----ATTP	527
	* *	
	NADPH	
HsMTRR	IIMVGPGGTGIAPFIGFLQHREKLQEHPDGNFGAMW-LFFGCRHKDRDYL	588
CeMTRR	LLMVGPGGTGVSVFLSFLHFLRKLKQDSPDFVDVPRVLFFGCRDSSVDAI	579
HsCPR	VIMVGPGGTGVAPFIGFIQERAWLRQQGKE--VGETLLYYGCRRSDEDYL	574
	..*****. * . * . * . * . *	
	NADPH NADPH	
HsMTRR	FRKELRHFLKHGILTHLKVFSRDAPVGEEEAAPAKYVQDNIQLHGQQVAR	638
CeMTRR	YMSELEMFVSEGILTDLIICESEQ-----KGERVQDGLRKYLDKVL	621
HsCPR	YREELAQFHRDGAALTQLNVAFSRE-----QSHKVYVQHLLKQDREHLWK	618
	. ** * * * * . * . *	
	NADPH	
HsMTRR	ILLQE-NGHIYVCGDAKNMAKDVEDALVQIISKEVGVKELEAMKTLATLK	687
CeMTRR	FLTASTESKIFICGDAKGMSKDVWQCFSDIVASDQGIPLDLEAKKKLMDLK	671
HsCPR	LI--EGGAHIYVCGDARNMARDVQNTFYDIVAELGAMEHAQAVDYIKKLM	666
	. * ..*****. * ..** . * * . *	
	NADPH/FAD	
HsMTRR	EEKRYLQDIWS	698
CeMTRR	KSDQYIEDVWG	682
HsCPR	TKGRYSLDVWS	677
	* * . *	

Fig. 4-4



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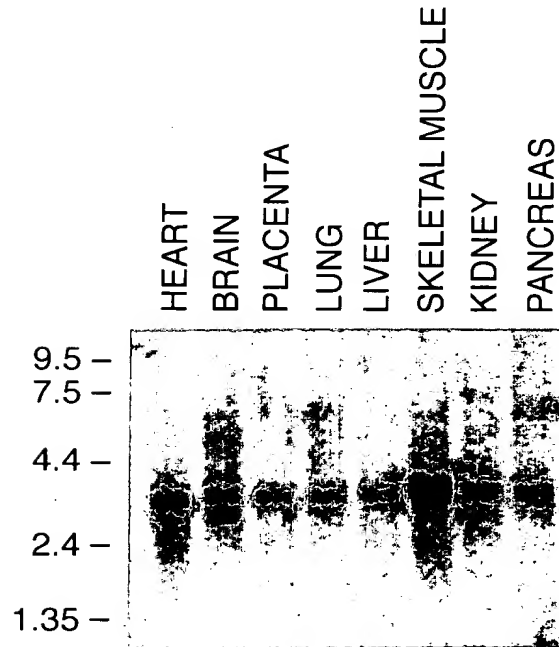
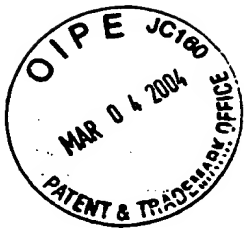


Fig. 5A



Fig. 5B



HUMAN METHIONINE SYNTHASE REDUCTASE: CLONING,
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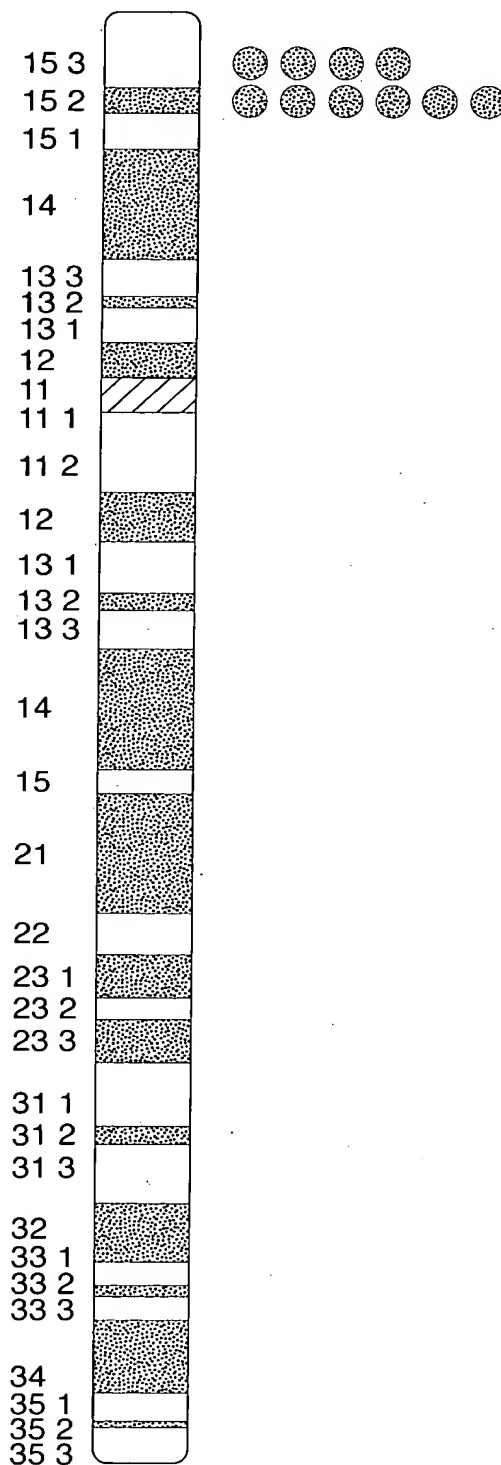
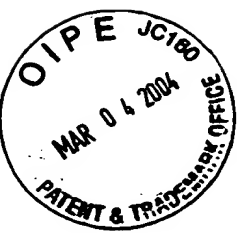


Fig. 6



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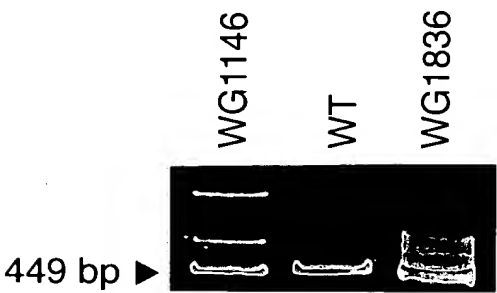


Fig. 7A

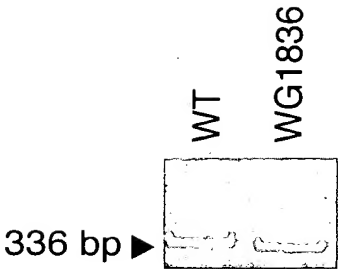


Fig. 7B

Position	Sequence	Accession#	Protein	Organism
572	GAMW [▲] LFFGCRHKDRDYLF	(AF025794)	MTRR	(H sapiens)
558	GETLLYYGCRRSDEYLY	(A60557)	CPR	(H sapiens)
559	GETLLYYGCRRAAEDYLY	(D00101)	CPR	(O cuniculus)
560	GESILYFGCRKRSEDYIY	(X93090)	CPR	(D melanogaster)
572	GPALLFFGCRNRQMDFIY	(P37116)	CPR	(V radiata)
573	GPTVLFFGCRKSDEDFLY	(Z26938)	CPR	(A niger)
1281	CPMVLVFGCRQSKIDHIY	(D16408)	NOS I	(H sapiens)
1009	GRMTLVFGCRRPDEDHIY	(U05810)	NOS II	(H sapiens)
1040	TPMTLVFGCRCSQLDHLIY	(L26914)	NOS III	(H sapiens)
380	GRMTLVFGCRHPPEEDHLIY	(U85094)	NOS	(O cuniculus)
1005	GDMILLFGCRHPDMDHIY	(U46504)	NOS	(G gallus)
481	GKNWLFFGNPHFTEDFLY	(M23008)	SR	(E coli)
915	GEVFLYLGSRHKREEYLY	(L26503)	SR	(S cerevisiae)
407	GRNWLIFGNRHFHRDFLY	(Z23169)	SR	(T roseopersicina)
261	GLAWLFLGVANVDSLLYD	(X99419)	FNR	(P sativum)
251	GLAWLFLGVPTSSSLLYK	(P00455)	FNR	(S oleracea)

Fig. 7C



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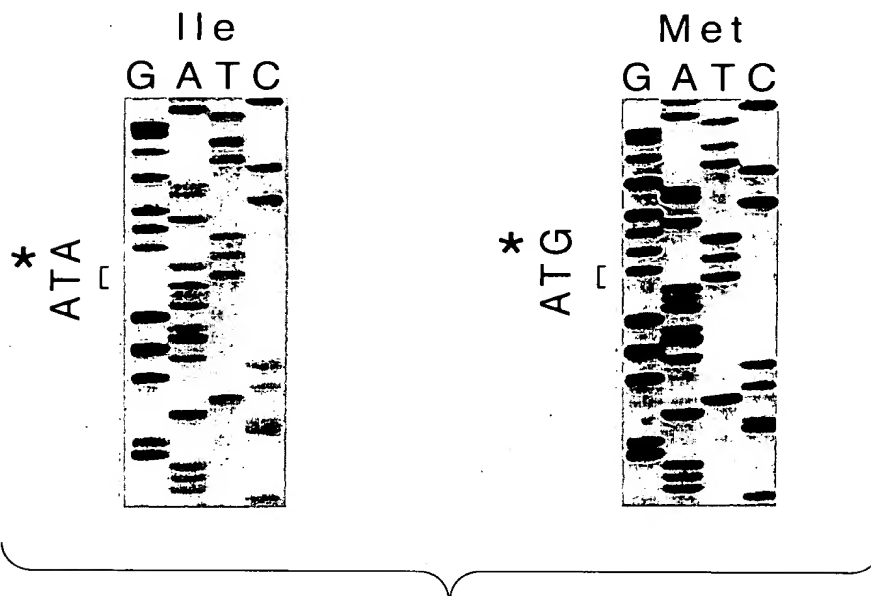


Fig. 8A



Fig. 8B